Clinical characterization of allergic sensitization patterns and the role of mucosal dendritic cells

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Chapter 4

Desloratadine Reduces Systemic Allergic Inflammation Following Nasal Provocation in Allergic Rhinitis and Asthma Patients

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Abstract

**Background:** Preclinical studies have demonstrated that some second-generation antihistamines have anti-inflammatory effects. It is not known whether these effects are also demonstrable *in vivo*. In this study we investigated the effect of treatment with desloratadine on systemic inflammation and on nasal and bronchial mucosal inflammation after nasal allergen provocation in subjects with grass-pollen-allergic rhinitis and asthma.

**Methods:** Twenty-six subjects with grass-pollen-allergic rhinitis and asthma were randomly allocated to eight days of treatment with desloratadine (n=13) or placebo (n=13) outside the grass pollen season. On day 7 they underwent nasal provocation with grass pollen allergen. Nasal and bronchial biopsies were taken for immunohistochemical evaluation, and blood samples were analysed. Rhinitis and asthma symptoms, peak nasal inspiratory flow, and peak expiratory flow were also measured at specified times.

**Results:** The number of circulating eosinophils decreased during desloratadine treatment, and there was a reduced increase in circulating eosinophils after nasal allergen provocation in these subjects. There was also a significant reduction in early bronchial clinical response. There was no significant lessening in the severity of the nasal symptoms. Nasal and bronchial mucosal inflammation parameters did not alter under desloratadine treatment.

**Conclusions:** These data suggest that treatment with desloratadine reduces systemic eosinophilia and prevents the increase in circulating eosinophils after nasal allergen provocation. Desloratadine also significantly reduces the early bronchial clinical response to nasal allergen provocation. However, airway mucosal inflammation is not altered by one week of treatment.
Introduction

The ‘one airway, one disease’ hypothesis has changed our view of the pathophysiology of allergic rhinitis (AR) and asthma: these two pathologic conditions should not be thought of as separate entities, but as different clinical manifestations of the atopic syndrome. Furthermore, subjects with AR have an increased risk of developing asthma (1).

The pathophysiological mechanism underlying AR and asthma is likely to be a similar inflammatory process, with eosinophils and mast cells acting as important effector cells. In patients with AR without asthma, nasal allergen provocation induces allergic inflammation of the bronchial mucosa in addition to the expected allergic inflammation of the nasal mucosa. Conversely, segmental bronchial provocation also leads to allergic inflammation in both nasal and bronchial mucosa. (2,3) The systemic pathway, which includes the bloodstream and bone marrow, contributes to the interaction between the upper and the lower airways, as allergen provocation results in an increase in circulating inflammatory cells and mediators. (2,4)

In this study, we investigated the efficacy of a seven-day course of treatment with the second-generation antihistamine desloratadine (DL) on inflammatory and clinical parameters of the upper and lower airways after nasal allergen provocation (NP) in subjects with grass-pollen-allergic rhinitis and asthma. By contrast with the older antihistamines, the second-generation antihistamines have been reported to be effective in the treatment of AR, including nasal obstruction, and also in reducing asthma symptoms and β2-agonist use in patients with AR and asthma. (5-9) The exact mechanism involved in this reduction of symptoms is not known. Earlier in vitro studies have not only shown that desloratadine acts as an H1 antagonist, but also that it has anti-inflammatory effects, including the inhibition of cytokines, chemokines, and adhesion molecules involved in systemic allergic inflammation. (10-12) Murine model studies have also shown that antihistamines inhibit bronchial inflammation and bronchial hyperresponsiveness by depressing cytokine production by T-cells. (13,14) One clinical study found a significant reduction of ICAM-1 expression on epithelial cells and a reduction trend in inflammatory cell counts in nasal scrapings taken from children after treatment with an antihistamine. (15) We hypothesised that DL could act at the level of important inflammatory effector cells, for instance by limiting a systemic eosinophilic response or by reducing the topical infiltration of eosinophils into the nasal or bronchial mucosa. To test this possibility, we analysed the dynamics of eosinophils (BMK-13), and looked at markers indicative of cellular migration into local tissue (Eotaxin, IL-5, and VCAM-1).
Methods

Subjects

The subjects selected for this study were adults with seasonal allergic rhinitis and asthma. All subjects had a history of grass pollen allergy (confirmed by skin-prick-test) with nasal and bronchial symptomatology for at least two years during the grass pollen season. At screening, they were tested for a panel of 10 common inhalant allergens (ALK-Abello BV, Nieuwegein, the Netherlands). A skin prick test was considered positive when the wheal diameter was 3 mm larger than that produced by the negative control after 15 minutes. Sensitisation to other allergens like dander or house-dust mite was allowed, as long as rhinitis symptoms were most pronounced during the grass pollen season. Asthma was diagnosed using ATS criteria (1987), and FEV₁ had to be over 60% of the predicted value or greater than 1.5 litres for safety reasons. All subjects suffered from wheezing, coughing or shortness of breath, and bronchial hyperreactivity (methacholine PC₂₀ <8 mg/ml). All subjects were non-smokers and in a stable clinical condition, i.e. no recent history of upper or lower respiratory tract infection, no acute asthma attacks, and no hospital admissions during the four weeks prior to investigation. Subjects were also excluded if they suffered from a disorder likely to interfere with the test results. All medication for allergic rhinitis and asthma treatment, and medication with possible interactions with desloratadine, was stopped before enrolment.

Study design

We performed a double-blind, placebo-controlled, parallel-group, exploratory study to evaluate the effects of desloratadine on upper and lower airway allergic inflammation following NP. The study was conducted outside the grass pollen season (2001/2002). Eligible subjects were randomly assigned to desloratadine or placebo for eight days in the form of one tablet (5 mg) each morning. The study drugs were provided by Schering-Plough Corporation (Kenilworth, NJ, USA).

The following nasal and pulmonary symptoms were recorded on a 100 mm visual analogue scale (VAS): rhinorrhoea, nasal itching, sneezing, nasal blockage, watery eyes, wheezing, coughing, shortness of breath, and decreased exercise tolerance. Symptom-VAS, PNIF, and PEF were recorded at baseline (day 0), on day 7 before NP and 15-30-45-60-90-120 minutes after NP, and 24 hours after NP (day 8).

Nasal and bronchial biopsies and blood samples were taken before the start of treatment (day 0), before NP after seven days of treatment (day 7), and 24 hours after NP (day 8). On day 7, the subjects were challenged with 10,000 BU/mL grass pollen extract (ALK-Abello, Nieuwegein, Netherlands) via a pump spray delivering a fixed dose of 89 mL into each nostril, one hour after nasal and bronchial biopsies were taken (Figure 1). All participants
Testing for obstruction of upper and lower airways

Obstruction of the upper and lower airways was tested on the basis of peak nasal inspiratory flow (PNIF), peak expiratory flow (PEF), and forced expiratory volume in 1 second (FEV1). PNIF was measured using an In-check inspiratory flow meter with face mask (Clement Clarke International Ltd., Harlow, England) and PEF using a Personal Best® peak flow meter (Respironics HealthScan, Cedar Grove, NJ, USA). The highest value of three measurements was recorded. FEV1 was determined by standard spirometry during each visit.

Methacholine provocation test

To measure bronchial hyperreactivity, a methacholine provocation test was performed during the run-in period and repeated on day 9. Methacholine was administered using a standardised tidal breathing method. The response to methacholine was measured as the change in FEV1 expressed as a percentage of the initial value. Records were kept of the provocative concentration of methacholine causing a 20% fall in FEV1 (PC20).
Blood samples and mucosal biopsies

Blood samples were collected before treatment started (day 0), after seven days of treatment and before NP (day 7), and 24 hours after NP. The total number of blood eosinophils was measured by means of a haemocytometric differential cell count. All biopsy specimens of the nasal mucosa were taken by the same investigator (SMR). First, local anesthesia was induced by placing a cotton-wool carrier with 50-100 mg of cocaine and three drops of epinephrine (1:1000) under the inferior turbinate, without touching the biopsy site. Then, a mucosal biopsy sample was obtained from the lower edge of the inferior turbinate about 2 cm posterior to the edge, using a Fokkens forceps with a cup diameter of 2.5 mm. Nasal biopsies were embedded in Tissue-Tek II Optimal Cutting Temperature (OCT) compound (Sakura Finetek USA Inc., Torrance, CA, USA), frozen and stored at -80 °C.

All bronchial biopsies were taken by the same pulmonary physician (SEO). After intramuscular pre-medication with atropine dinitrate (0.5 mg), oropharyngeal anesthesia was accomplished with topical xylocaine spray 1%. Next, the vocal cords, trachea and bronchial tree were anaesthetized with oxybuprocaine. The fibreoptic bronchoscope (BF, type P20 D; Olympus, Tokyo, Japan) was introduced into the airway via the oral route and mucosal biopsies were taken from the segmental carinae of the left and right lung. Bronchial biopsies were embedded in Tissue-Tek II OCT compound, frozen and stored at -80 °C. (3)

Immunohistochemical staining and microscopic assessment

The monoclonal antibodies (mAbs) used in this study were BMK-13 (IgG1, 0.2 μg/mL, Sanbio, Uden, the Netherlands) for identifying eosinophils, IL-5 (3 mg/mL, IgG1, kindly provided by Prof. J. Tavernier, Ghent, Belgium), Eotaxin (500mg/ml, IgG1, R&D, Minneapolis, USA), and VCAM-1 (100 mg/ml, Monosan Sanbio, IgG1, Uden, Netherlands). The mAb stainings were developed with the supersensitive immoninkalike phosphatase (ss-AP) method described elsewhere. (17) Biopsies were coded and counted blind for each antibody using a method described elsewhere. (3) Positively stained inflammatory cells localised in epithelium and subepithelium (area 100 μm into the lamina propria along the length of the epithelial basement membrane) were counted along the basement membrane (BM), using an Axioskop 20 microscope (Zeiss, Jena, Germany) with an eyepiece graticule at a magnification of x 200. Cell numbers were expressed as positively stained cells per mm BM.

Statistical analysis

The Wilcoxon signed-rank test for within-group analysis and the Mann-Whitney U test for between-group analysis were performed. In the case of repeat measurements, we determined the area under the curve and compared the groups using the Mann-Whitney
U test. Data are presented as medians and ranges or mean ± SD, as indicated. A p-value of less than 0.05 was considered significant.

**Results**

**Subject characteristics**

Twenty-six eligible subjects (14 women, mean age 30.6 yrs ±10.7) were allocated randomly to treatment with either desloratadine or placebo. Five subjects dropped out of the study before nasal allergen provocation because they were unable to comply with the study protocol. The remaining 21 subjects – 10 in the desloratadine group and 11 in the placebo group – were comparable in terms of demographic and clinical parameters at baseline (Table 1).

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Desloratadine</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Sex (% female)</td>
<td>55 ± 16</td>
<td>50 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>31.7 ± 3.6</td>
<td>31.6 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1 (%pred)</td>
<td>102.7 ± 4.7</td>
<td>99.0 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>108.1 ± 3.8</td>
<td>103.7 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1a (%pred)</td>
<td>107.6 ± 3.9</td>
<td>103.3 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>FVCa (%pred)</td>
<td>106.1 ± 3.8</td>
<td>105.4 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>TNSS (mm)</td>
<td>20.5 ± 7.8</td>
<td>43.8 ± 12.6</td>
<td>NS</td>
</tr>
<tr>
<td>TBSS (mm)</td>
<td>19.0 ± 6.9</td>
<td>54.7 ± 27.5</td>
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</tr>
<tr>
<td>PNIF (L/min)</td>
<td>167.7 ± 22.3</td>
<td>122.0 ± 14.7</td>
<td>NS</td>
</tr>
<tr>
<td>PEF (L/min)</td>
<td>490.0 ± 34.1</td>
<td>465.0 ± 39.5</td>
<td>NS</td>
</tr>
<tr>
<td>PC20 Meth (mg/ml)</td>
<td>2.00 ± 0.61</td>
<td>2.31 ± 0.41</td>
<td>NS</td>
</tr>
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</table>

FEV1 = forced expiratory volume in the first second  
FVC = functional vital capacity  
FEV1a = FEV1 after bronchodilatation  
FVCa = FVC after bronchodilatation  
TNSS = total nasal symptom score  
TBSS = total bronchial symptom score  
PNIF = peak nasal inspiratory flow  
PEF = peak expiratory flow  
PC20 Meth = methacholine provocation test, concentration resulting in 20% reduction of FEV1

Desloratadine treatment improves nasal patency, but not clinical bronchial parameters

During treatment in the week prior to nasal provocation, we observed a substantial increase in PNIF in every single subject in the desloratadine group, and only a limited increase in six of the eleven subjects in the control group. The median increase in PNIF was 60 L/min
in the DL group, significantly higher than the increase of 10 L/min in the placebo group (p=0.01). We did not find significant changes in the clinical bronchial parameters during the seven-day treatment period prior to nasal allergen provocation. Methacholine PC20 thresholds before treatment were the same for the desloratadine group (median 2.18 mg/mL, range 0.60 – 7.2 mg/mL) and the control group (median 0.85 mg/mL, range 0.21 – 5.6 mg/mL) and remained constant in both treatment modalities (desloratadine: median 2.15 mg/mL, range 0.88 – >39.3 mg/mL, and controls: median 0.98 mg/mL, range 0.17 – 16.7 mg/mL). The PC20 methacholine threshold in one patient in the desloratadine group improved dramatically (0.94 mg/mL before treatment, and >39.3 mg/mL after). Overall, there was no change in the methacholine threshold before and after treatment in either group. There were also no significant differences between the two groups in FEV1 throughout the study (data not shown).

Desloratadine reduces the effect of nasal provocation on bronchial symptoms and signs, however nasal symptoms were not reduced

During the first two hours after nasal allergen provocation, all individual nasal symptom scores increased as expected. The maximum increase in total nasal symptom score was reached after 15 minutes. The median scores after 15 minutes were 136 mm in the placebo group and 88 mm in the desloratadine group. The median areas under the curve (AUC) of the total nasal symptom score during the first two hours after NP were 118 mm and 104 mm respectively. However, these differences between DL and placebo did not attain statistical significance. Nor did we observe a significant improvement in PNIF during the first two hours after NP in the desloratadine group compared to the placebo group. Despite the limited effects we observed on nasal symptoms and PNIF, the effect of DL on the early bronchial symptoms and signs was evident. After NP, the increase in the total bronchial symptom scores was significantly lower in the desloratadine group than in the control group (p=0.05; Figure 2). The median AUCs for the total bronchial symptom score during the first two hours after NP was 10 mm in the placebo group and –18 mm in the treated group. In addition, we observed that PEF tended to be better in the subjects treated with DL (p=0.09; Figure 3) than in controls. The median decreases in PEF expressed as AUC during the first two hours after NP were 32.5 L/min in the placebo group and 10 L/min in the desloratadine group. The maximum decrease in PEF was observed after 45 minutes; the median decrease after 45 minutes in the placebo group was 30 L/min compared to 20 L/min in the desloratadine group.

Desloratadine treatment reduces the number of circulating eosinophils, but not the airway mucosal eosinophilia

The clinical data revealed that a seven-day course of treatment with DL resulted in a significant improvement in nasal patency. This observation was matched by a reduction
DL reduces systemic allergic inflammation

The number of circulating eosinophils. In the placebo group, the median percentage of eosinophils was 2.0%. This percentage remained constant during treatment, whereas in the DL group the median percentage of eosinophils fell from 2.5 to 1.5% (Figure 4). This reduction was poorly reflected in the dynamics of the number of eosinophils in the nasal mucosa. At baseline, low numbers of eosinophils were detected in the nasal lamina.

Figure 2. The total bronchial symptom score after NP was significantly better in the subjects treated with desloratadine (p=0.05)

Figure 3. After NP the reduction in PEF tended to be less in the subjects treated with DL (p=0.09)
propria (median number 1.6/mm BM, range 0.0 – 7.4/mm BM in the placebo group, and median number 1.6/mm BM, range 0.0 – 18.3/mm BM in the desloratadine group). Eosinophil counts were not significantly reduced by DL treatment (median number 0.7/mm BM, range 0.0 – 37.9/mm BM). These observations concur with the data about the effect of DL treatment on the number of blood vessels staining positive for VCAM-1 and the number of local nasal IL-5 and eotaxin positive cells. However, we did not observe any significant changes with DL treatment (data not shown).

The median number of eosinophils in the bronchial mucosa was reduced from 24.3/mm BM (range 0.7 – 33.3/mm BM) to 4.7/mm BM (range 0.0 – 30.0) in the DL group. The median number of eosinophils in the placebo group was only moderately reduced from 7.5/mm BM (range 0.5 – 36.0/mm BM) to 4.0/mm BM (range 0 – 9.4/mm BM). The changes in the median number of eosinophils in the bronchial mucosa did not reach statistical significance. The number of VCAM-1-positive vessels in the bronchial mucosa mirrors these effects on the eosinophils. However, these results were not significant. We observed a decrease in VCAM-1-positive vessels in the treated group from 22.8% (range 0-100%) to 0% (range 0-12%). There were no changes (median 0%, range 0-56% before, and median 0%, range 0% after treatment) in VCAM-1-positive vessels in the placebo group.

Figure 4. In the placebo group blood eosinophils significantly increased after NP (p=0.01). After a seven-day course of desloratadine systemic eosinophilia is reduced, and than increases after NP, however overall there are no significant changes.
Desloratadine reduces the impact of eosinophilic response after nasal provocation

Nasal allergen provocation induced a significant increase – from 2.0% to 4.0% (p=0.01) – in the number of circulating eosinophils in the placebo group. This increase was prevented in the desloratadine group. Overall, the number of eosinophils did not change significantly in the desloratadine group, showing that a strong activation of systemic inflammation after NP was prevented in the DL group (Figure 4).

The local effect of desloratadine after strong nasal allergen provocation seems to differ in the nasal and bronchial epithelia. Although all patients had a positive skin prick test for grass pollen and a history of allergic rhinitis, nasal allergen provocation with grass pollen allergen resulted in a substantial increase of the number of eosinophils in the nasal mucosa in only a limited number of patients (4 out of 11 in the placebo group, and 4 out of 10 in the DL group (Figure 5). There were no significant differences in the increase in nasal mucosal eosinophilia between the placebo and the DL group, despite the reduced increase in the number of circulating eosinophils observed after NP in the DL group. Our data show that this discrepancy cannot be explained by differential up-regulation of the number of VCAM-1-positive vessels or the number of IL-5 and eotaxin positive cells in the nasal mucosa.

![Graph](image)

**Figure 5.** Nasal allergen provocation with grass pollen allergen resulted in a substantial increase of the number of eosinophils (number of BMK13+ cells/ mm BM) in the nasal mucosa in only a limited number of patients (measured before, and 24 hours after, NP)
mucosa, as there is no significant up-regulation of these numbers after provocation (data not shown). By contrast with the restricted up-regulation of the number of eosinophils in the nasal mucosa, no increase was observed in local eosinophilia in the bronchial mucosa. Nor did the number of VCAM-1-positive vessels or the number of IL-5 and eotaxin-positive cells change after nasal allergen provocation (data not shown).

**Discussion**

The aim of this study was to investigate whether the *in vivo* effects of antihistamines on nasal and bronchial symptomatology could be attributed to the anti-inflammatory properties of these drugs. The main outcome of our study is that desloratadine does indeed have an *in vivo* effect on allergic inflammation after a seven-day course of treatment in patients with allergic rhinitis and asthma. Systemic eosinophilia is significantly reduced after a seven-day course of treatment. The increase in eosinophilia normally observed after nasal allergen provocation is also lessened. As the systemic pathway plays an important role in the interaction between the upper and the lower airways (18), desloratadine may prevent the up-regulation of inflammatory cells and mediators in the lower airways after a stimulus in the upper airways. This reduction of systemic eosinophilia was accompanied by an improvement in nasal patency as measured by PNIF. No full explanation can be provided of the symptoms at baseline in our study group. Nasal symptoms could be a consequence of either minimal persistent disease related to the allergic rhinitis or to the effect of asthma on upper-airway symptomatology. As the improvement in PNIF was comparable for the subjects with and without sensitisation to house-dust mite, we assume that baseline nasal symptoms are a consequence of the interaction between lower and upper airways.

By contrast with our observations of the effects of desloratadine on systemic eosinophilia, no clear effect of this kind was observed in the nasal or bronchial mucosa. Despite the increase nasal and lung symptomatology, there was a substantial increase in the number of eosinophils in the nasal mucosa in a limited number of patients only, and there was no increase in eosinophil numbers in the bronchial mucosa. On the basis of previous findings by Braunstahl et al (2), we expected a significant increase in eosinophils in both nasal and bronchial mucosa. However, they studied a group of grass-pollen-allergic rhinitis subjects without asthma. Our population, with rhinitis and asthma symptoms and multiple sensitisations, is in a more advanced stage of the allergic disease, and may have developed tolerance mechanisms reducing the inflammatory response to a similar stimulus. Our findings are in line with earlier reported data that mucosal eosinophilia is less marked in perennial rhinitis than in seasonal rhinitis, and usually unrelated to clinical symptoms (19). We did not find a significant difference in tissue eosinophilia between the placebo and the desloratadine groups. We hypothesise that treatment with desloratadine cannot reduce the number of eosinophils present in the mucosa, but may prevent the influx of
eosinophils into the tissue due to the reduction of circulating eosinophils. A longer period of treatment would be necessary to confirm this hypothesis. The discrepancy between treatment effects on eosinophilia in the systemic and mucosal compartments has also been observed in the experimental treatment of asthma with an antibody targeting IL-5. In the systemic compartment, this treatment resulted in a marked reduction of eosinophils, whereas the reduction of eosinophils in the tissue was rather modest (Bachert C, personal communication).

The lack of local response may also be explained by the difference in desloratadine concentration in the systemic or local compartment. An in vitro study showed that the anti-inflammatory effect of desloratadine was only demonstrable at concentrations greater than achieved in the serum. Thus, the inhibition of allergic inflammation by desloratadine may well be dose-dependent.

The question of whether antihistamines have an anti-inflammatory effect is very relevant in clinical terms. In persistent allergic rhinitis and in asthma, the chronic nature of the disease requires life-long medical treatment for the affected patients. The application of local corticosteroids in the treatment of allergic rhinitis and asthma has been shown to be very effective in reducing signs and symptoms and also in circumventing some of the side-effects associated with the use of systemic corticosteroids. However, the prolonged use of inhaled steroids or high doses may still lead to systemic side-effects. In the treatment of allergic rhinitis and asthma, second-generation antihistamines have proven to be a good alternative with very limited side-effects. The efficacy, safety and pharmacology of desloratadine have been assessed according to the ARIA/EAACI criteria for antihistamines, and were found to broadly meet these criteria.

Our findings are in line with these previous observations: we observed a diminished allergic response to nasal allergen provocation in patients with allergic rhinitis and asthma. One week of desloratadine treatment significantly reduced the early bronchial clinical response to NP during the first two hours after provocation.

We conclude that desloratadine reduces systemic eosinophilia and can prevent the increase in systemic eosinophilia after nasal allergen provocation. DL also significantly reduces the early clinical bronchial response to nasal allergen challenge. We did not observe a significant effect on local nasal and bronchial mucosal inflammation parameters. We hypothesise that a longer treatment period is necessary to observe a decrease in the number of eosinophils in airway mucosa.

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